Title

DEVICE FOR THE ATOMIZATION OF LIQUID SAMPLES

Field of the Invention

The invention is directed to a device for atomizing liquid samples for spectroscopic measurements, comprising a tubular furnace which has a flame-heated tube, and a device for introducing a sample into the flame-heated tube. In flame atomic absorption spectrometry (flame AAS), the device according to the invention leads to a detection capacity that is appreciably improved over the prior art, wherein particular advantages are given with respect to the handling of microsample quantities.

Prior Art

In flame AAS, the sample introduction is usually carried out via an aerosol which is introduced into the flame and which is generated by means of a pneumatic atomizer. The usable portion of this aerosol generally amounts to only about 5%, so that valuable sample material is not utilized and is lost and, further, the detection capacity of the method can not be fully utilized. The sample enters the flame area together with the flame gases which are premixed in the atomizer chamber, so that the holding period in the absorption volume is very short. The low atomizer yield and the short holding period of the sample in the absorption volume result in a detection capacity which is not sufficient for many analytic tasks.

In addition to this standard sample introduction via pneumatic atomizers, a large number of special techniques are described in the technical literature. An overview of the prior art of atomic spectrometric sample introduction is given, for example, in monographs by B. Welz and M. Sperling, "Atomabsorptionsspektrometrie [Atomic Absorption Spectrometry]", Wiley-VCH, (1997), ISBN 3-527-28305-6, and by A. Montaser, "Inductively Coupled Plasma Mass Spectrometry", (1998) Wiley-VCH, ISBN 0-471-18620-1.

A known special sample introduction for atomic spectrometry, especially for ICP spectrometry, is aerosol generation via a thermospray arrangement. In this connection, the liquid is generally guided through a directly or indirectly electrically heated, temperature-regulated capillary, wherein a portion of the liquid is evaporated. The occurring steam propels the front of the liquid out of

the capillary as aerosol. This thermospray capillary (TS capillary) replaces the pneumatic atomizer. The heated length is usually between 10 cm and 50 cm, wherein heating is carried out uniformly along the entire length.

Known TS arrangements work in a temperature range between 140° C and 360° C. Because of the high flow resistance, the liquid transport is normally carried out with a high-pressure liquid chromatography pump (HPLC pump). An overview of the operation and use of the thermospray in atomic spectrometry is given in two articles: a) J. A. Koropchak and M. Veber, "Thermospray Sample Introduction to Atomic Spectrometry", Critical Reviews in Analytic Chemistry, 23, 113-141 (1992), b) T. S. Conver, J. Yang and J. A. Koropchak, "New Developments in thermospray sample introduction for atomic spectrometry", Spectrochimica Acta, Part B, 52, 1087-1104 (11997).

Another known special sample introduction in atomic spectrometry is based on the principle of hydraulic high-pressure atomization as is described in patent DE 3521529 C2. The liquid to be atomized is converted to aerosol by means of a special high-pressure atomization nozzle with a flow cross section of less than $1.5 \times 10^{-9} m^2$ and with a pressure of more than 3 MPa, wherein the pressure is generated with a commercially available high-pressure liquid chromatography pump (HPLC pump).

In an application example of hydraulic high-pressure atomization, the aerosol spray generated by the high-pressure nozzle is introduced into a flame-heated ceramic tube along a distance of several centimeters. An increase in sensitivity of about twenty times was achieved for determination of traces of lead (H. Berndt, Fresenius J. Anal. Chem., vol. 331, (1998), pp 321 - 323). This increase in sensitivity is essentially based on a holding period of the sample in the measurement volume that is longer than in the usual AAS measurements and on the introduction of the sample into the tube without losses.

In contrast to the introduction of gaseous samples, however, the introduction of liquid samples into a flame-heated glowing tube continues to be regarded as unresolved and difficult because the usual sample introduction techniques in analytic chemistry cannot be applied due to the high temperature. For

instance, approaching with a dosing pipette made predominantly of plastic would immediately result in a total destruction of the pipette.

The use of flame-heated measurement cells, predominantly T-shaped quartz tubes, for atomic absorption spectrometric measurements is likewise prior art. The best known example consists in the widely applied hydride techniques, wherein the gaseous hydrides of the hydride-forming elements (e.g., As, Se, Te, Sb) are introduced into the hot cell. The hydrides are decomposed in the tube and the elements are measured by atomic absorption spectrometry. The introduction of the gaseous compounds into the glowing tube is carried out via the lateral extension piece of the tube. The flame-heated tubes are being replaced to an increasing extent by indirectly electrically heated tubes. The differences in the various measurement cells consist essentially in the type of heating and in details of tube geometry. The hydride technique is extensively described in all monographs relating to atomic absorption spectrometry. One example is the comprehensive book by B. Welz and M. Sperling, "Atomabsorptionsspektrometrie [Atomic Absorption Spectrometry]", Wiley-VCH, (1997), ISBN 3-527-28305-6.

Due to the widespread practice of introducing gaseous compounds into a glowing tube, there are relatively many publications in this regard, some of which may be mentioned and briefly cited in the following.

GB 14 25 188 describes a flame-heated tube with lateral connection in which gaseous compounds are introduced. DE 26 40 285 C2 discloses an arrangement comprising a reaction vessel and a heated measurement cuvette in which gaseous compounds are formed in the reaction vessel by chemical reactions (with the addition of reagents) and are then introduced into a heatable measurement cuvette.

DE 27 45 148 B2 describes a modification of a measurement cuvette of the kind used in the above-mentioned applications GB 14 25 188 and DE 26 40 285 C2. In this case, the tube has a connection in its middle region for introducing a measurement gas.

DE 28 05 137 C2 shows a device for automatically supplying liquid to a pneumatic atomizer of an atomic absorption spectrometer. As is conventional, the liquid is atomized in a chamber from which only a small portion of the aerosol is

then transported into the flame. Accordingly, this is a normal flame atomic absorption measurement arrangement with automatic sample feed. Its only peculiarity consists in that the sample is deposited in a funnel from which it is sucked in by the atomizer. In this way, microsamples can be delivered automatically also in flame AAS.

In DE 35 21 529 A1, hydraulic high-pressure atomization is used as a special type of atomization. In this connection, a liquid sample is atomized via a high-pressure nozzle with a minimum pressure of 30 bar. While the usable portion of aerosol is higher than in other kinds of atomization, e.g., pneumatic atomization, the improved detection capacity achieved in this way is often not sufficient for determining small element trace concentrations which commonly occur in routine analysis.

Flame-heated tubes are also used in atom trapping techniques, as they are called. In this case, the sample is fed to the burner head of the AAS spectrometer via a pneumatic atomizer and a gas mixing chamber as is conventional. A slit quartz tube in which a portion of the flame is held back for a brief period is arranged on the burner head. In this way, a relatively slight improvement in detection capacity is achieved for about six elements. A typical example from the abundant original material on atom trapping techniques is that of R. Keil, "Verbesserung der Empfindlichkeit in der Flammen-Atomabsorptions-Analyse durch Einsatz eines Quarzrohres mit Längsschlitz in den Atomisierungsraum [Improved Sensitivity in Flame Atomic Absorption Analysis through the Use of a Longitudinally Slit Quartz Tube in the Atomization Chamber]", Fresenius Z. Anal. Chem. (1984) 319, pp391-394. An extensive overview of this technique is given in an article by H. Matuslewicz, Spectrochimica Acta B, 52 (1977) 1711-1736.

Description of the Invention

Proceeding from the prior art described above, it is the object of the invention to overcome the problems in sample introduction caused by temperature and to supply the liquid samples to a flame-heated tubular furnace discontinuously or continuously in a highly efficient manner.

According to the invention, a device of the type mentioned above has a sample inlet opening in the tube to which a capillary leads, the capillary is flame-heated at its tube-side end along with the tube, and a pump is provided for delivering a liquid sample through the capillary, wherein the sample is partially or completely evaporated in the capillary, which acts as a thermospray, and flows into the tube in this state.

The capillary and the flame-heated tube are so positioned with respect to a burner head from which the heating flame exits that the capillary as well as the tube are heated by the flames.

Due to the fact that the thermospray principle is rendered usable for flame atomic absorption spectrometry in this way, the introduction of liquid samples into a glowing hot tube is carried out discontinuously and continuously without problems and in a highly efficient manner. In this way, the entire sample substance is introduced directly into the measurement volume and a substantially higher efficiency is accordingly achieved compared with sample introduction via a pneumatic atomizer with atomizer chamber.

It is especially advantageous when the tube is positioned in such a way that it is heated along its entire length. It can also be advantageous to lower the intense temperature gradients at the furnace-side end of the capillary in order to achieve a uniform evaporation, for example. This can be achieved, for example, in that the capillary is enclosed along several centimeters of its length by a metal cylinder having a larger diameter. In this way, a larger portion of the capillary is heated and the temperature gradient is reduced via the heat conductivity of the metal jacket.

For this purpose, a capillary with the greatest possible resistance to flame gases and acids at high temperatures is introduced into a bore hole located on the side of the tubular furnace, wherein the best possible contact between the capillary and tubular furnace has proved to be advantageous. In this way, the end of the capillary is heated by the flame gases as well as by a direct heat transmission from the tubular furnace to the capillary. This type of heating dispenses with the separate heating of the capillary required in thermospray techniques. The liquid to be atomized and evaporated is supplied to the capillary via a pump, wherein even a

low-pressure pump, e.g., a peristaltic hose pump, provides a sufficient delivery pressure because of the low flow resistance in this arrangement.

In a preferred construction of the invention, the capillary and the tube are fixedly connected with one another mechanically. A construction in which the capillary is connected with an additional heating source, e.g., electric heating, is also possible.

The capillary should be constructed with an inner diameter between 0.02 mm and 2 mm and should be made of a metal with extensive resistance to chemicals and temperature, an alloy with extensive resistance to chemicals and temperature, ceramic or silica glass. With cylindrical capillaries of silica glass or ceramic, it may be advantageous when they are enclosed by a cylindrical jacket of metal or alloy.

The flame-heated tube of the tubular furnace should be manufactured from a metal with extensive resistance to chemicals and temperature, an alloy with extensive resistance to chemicals and temperature, ceramic or silica glass.

In special, likewise advantageous constructions of the invention, the burner head is constructed as a slit burner and the pump is formed as a continuously pumping peristaltic single-channel or multichannel pump, as a gas pressure pump, piston pump or diaphragm pump.

If a diaphragm pump is provided, it is recommended that it is followed by a pulsation damper. This eliminates the disadvantage of a pulsating liquid flow which is characteristic of diaphragm pumps. A very simple pulsation damper can be made, e.g., of a highly elastic silicon hose with a volume of about 100 ml, followed by a restrictor with a counter-pressure of 6 bar, e.g., a fine capillary hose or a valve. A diaphragm pump with a pump capacity of about 100 ml/min at a delivery pressure of 6 bar was tested, for example. The main quantity of liquid is returned to the suction side of the pump in a return via the silicon hose and the restrictor capillary (acting as work resistance). A T-piece for splitting the liquid flow is located between the silicon hose and restrictor capillary. At this location, the delivery quantity required for the thermospray system is adjusted to the desired flow rate, e.g., 1 ml/min, by another restrictor, for example, a piece of capillary hose.

It further lies within the framework of the invention that a sample feed device in the form of a manual sample feed valve or a sample changer with an automatic sample feed valve is arranged between the pump and capillary. The sample changer can be coupled with a control circuit for time-controlled sample changing.

Further, a partition or enrichment column can be inserted between the sample feed device and the capillary. Besides the sample inlet opening and two end openings, the flame-heated tube of the tubular furnace can be provided with at least one additional opening oriented in the direction of the burner slit. Further, in constructions lying within the scope of the invention, the flame-heated tube has, apart from the sample inlet opening and the two end openings, at least one additional opening facing away from the burner slit. A partial transverse flow of flame gases through the tube is achieved in this way.

Brief Description of the Drawings

Some embodiment examples of the invention will be described more fully with reference to the following drawings:

Fig. 1 shows a schematic view of the construction of a tested, automatic thermospray sample introduction in a flame-heated tubular furnace;

Fig. 2 shows an arrangement similar to Fig. 1 in which the sample is injected into a carrier flow on the pressure side of the pump by means of a sample feed valve;

Fig. 3 shows an HPLC sample changer replacing the manual sample feed valve for automatic sample feed;

Fig. 4 shows an arrangement in which the sample liquid is injected into the carrier flow by means of a sample feed valve, wherein a gas pressure pump is used for liquid transport.

Fig. 5 shows an arrangement similar to Fig. 4, but in which an HPLC pump is used;

Fig. 6 shows a section from Figs. 2, 3, 4 or 5, wherein a (low-pressure) partition column or enrichment column is inserted between the sample feed valve;

Fig. 7 shows an embodiment form of the flame-heated tube with additional bore holes;

Fig. 8 shows the signals for determination of cadmium from small sample quantities with an arrangement according to the invention;

Fig. 9 shows a sensitivity comparison for lead determination between a measurement arrangement according to the invention and conventional flame AAS.

Detailed Description of the Drawings

Fig. 1 is a schematic view showing the construction of a tested, automatic thermospray sample introduction into a flame-heated tubular furnace. A standard gas mixing chamber of a flame atomic absorption spectrometer is designated by 1. The associated AAS slit burner head is designated by 2 and has a burner slit 3 having a length of approximately 10 cm, an adjustable holder 4 carrying two pins 5 being mounted in each instance at the two sides of the burner slit 3. The tube 6 which has a bore hole 7 in its center lies on the pins 5. The bore hole diameter is also about 1/16 mm, for example. The end of a standard high-pressure liquid chromatography capillary (HPLC capillary) 8 having a length of about 15 cm and an outer diameter of 1/16" (1.58 mm) is pressed into this bore hole 7. The end of the capillary can terminate flush with the tube interior or can project into the tube interior by a distance of about 1 mm. An advantageous inner diameter of the capillary is 0.6 mm. In the simplest case, it can be an HPLC high-grade steel capillary. The capillary is heated directly by the flame of the slit burner head and by

the heat transmission at the press fit. The end of the capillary accordingly reaches the same temperature as the tubular furnace. The rest of the capillary is heated by the heat conductivity of the capillary material (heat conductivity), wherein the liquid which is transported in the interior of the capillary acts as counter-flow cooling. For this purpose, the capillary 8 has, on the liquid inlet side, a temperature that is only slightly higher than the ambient temperature. As it is transported through the capillary, the liquid is heated and is partially or completely evaporated and arrives in the tubular furnace as a liquid aerosol or as superheated steam. In terms of function, this capillary can be regarded as a special embodiment form of a thermospray which is characterized in that the heating is carried out only at one end and is not carried out over the entire length of the capillary as is conventional, in that the capillary does not require its own heating device, and in that, further, temperatures substantially higher than 500° C (e.g., about 900° C) are reached. Commercially available connection means which are used in analytic high-pressure and low-pressure flow systems (for example, hose connectors (1/16" - 1/16"), thin plastic hoses) are designated by 9. A typical laboratory hose pump (peristaltic pump) 10 is connected on its primary side with the suction needle 14 of a commercially available sample changer 15 via a thin hose 13. The pumping capacity is determined by the rotational speed of the pump head 11 and by the diameter of the pump hose 12. The needle 14 of the sample changer dips into the sample vessel 16 in a time-controlled manner. The received sample quantity is determined at a predetermined constant pumping capacity of the pump via the immersion time of the needle. The individual samples are separated from one another by an air cushion in this arrangement (segmented flow technique) so that the samples reach the (thermospray) capillary 8 without being diluted (without dispersing in a carrier flow). This results in a higher sensitivity in AAS measurement, particularly in sample volumes of only a few microliters.

The connection means 9 can be dispensed with when the cold end of the capillary 8 can be pressed directly into the pressure-side end of the delivery hose 12 of the pump 10. This was determined in a tested arrangement.

Instead of connecting the capillary 8 and tubular furnace 6 by a press fit, a more easily exchangeable connection can also be selected. A capillary with a

sealing cone of metal (ferrule, a standard HPLC connection accessory) attached at a distance of 2 mm from the end of the capillary was tested, wherein the diameter of the inlet opening 7 of the tubular furnace was 2 mm and was widened conically. However, the capillary can also be introduced into the tubular furnace in a noncontacting manner. This is achieved, for example, with a 0.9 mm capillary (0.6-mm inner diameter) with an inlet opening of the tubular furnace of 2 mm. However, this could also be a fixed connection between the tubular furnace and the capillary (T-piece).

A capillary 8 with a high resistance to acids which is made of a Pt/Ir alloy, e.g., 75% Pt / 25% Ir is advantageous with standard HPLC dimensions (e.g., 1/16" outer diameter, 1mm inner diameter). Capillaries of this type are specially manufactured, e.g., by Degussa, Frankfurt a. M., and by Hereaus, Hanau (tested construction).

Capillaries of silica glass (quartz glass), while resistant to acids, deform when acted upon by flame gases. TS arrangements with silica glass capillaries are known, wherein the silica glass capillary is enclosed by an electrically heated high-grade steel capillary (see above-cited article by Koropchak, et al.). A silica glass capillary can also advantageously be used for the arrangement according to Fig. 1 when it is enclosed by a high-grade steel capillary for protection against the indirect action of flame gases and for heat transmission. The enclosed (outer) capillary can be a piece of standard HPLC capillary of high-grade steel (e.g., 1/16" outer diameter and 0.3 mm to 1 mm inner diameter, wherein the inner diameter of the jacket capillary is determined by the outer diameter of the silica glass capillary located within it). The capillary can also comprise a ceramic which is highly resistant to temperature and acids. Capillaries of this kind are used, for example, for wire insulation in thermal elements. The ceramic capillary can also be enclosed.

Instead of supplying samples via a sample changer 15, samples can also be delivered manually. For this purpose, the sample is sucked in directly by the pump 10 via a hose 12 from a sample supply vessel.

With the arrangement according to Fig. 2, the supplying of samples is carried out by means of a sample injection valve 18 located between the pump 17 and the capillary 8. The pump 17 continuously sucks a carrier liquid 21 from a

supply vessel 19 via a hose connection 20 and transports it on the pressure side via connection means 9 to valve 18 and then on to the capillary 8. The carrier liquid 21 is water, for example. However, it could also be an organic solvent or mixtures thereof with water. By switching the valve 18, the sample contained in the sample hose 22 is embedded in the carrier flow and conveyed along with the latter to the capillary 8. The aerosol occurring in the capillary flows into the tubular furnace 6 corresponding to the description of Fig. 1. The sample quantity is predetermined by the dimensions of the sample hose 22. When connection means 9 having low dead volume are used, e.g., HPLC PEEK capillaries with a small inner diameter, the sample reaches the capillary 8 in substantially undiluted state.

Typical flow injection accessories for on-line sample preparation, e.g., microcolumns, can be added between the valve 18 and the capillary 8. Instead of the single-channel hose pump 17, a multichannel hose pump can also be used. However, two or more single-channel pumps whose liquid flows are combined prior to the capillary 8 can also be used. In this way, many known couplings of flow injection techniques or continuous flow techniques which were previously carried out via the pneumatic atomizer with its low efficiency., e.g., partition, enrichment, automatic calibration, can be utilized in a considerably improved manner with flame AAS. An overview of the many coupling techniques is contained, e.g., in the following books: a) J. L. Burguera, "Flow Injection Atomic Spectrometry" (1989), Marcel Dekker, Inc., New York, ISBN 0-8247-8059-0; b) J. Ruzicka and E. H. Hansen, "Flow Injection Analysis", (1988), John Wiley Sons, New York, ISBN 0-471-813555-9.

Fig. 3 shows a schematic view of an arrangement in which a commercially available HPLC sample changer 23 having an integrated automatic switching valve 24 is used instead of the manual sample feed valve 18 from Fig. 2. The sample hose 25 of this valve is filled by means of a spray pump contained in the sample changer. The carrier flow flows from the pump 17 (Fig. 2), via connection means 9, to the valve 24 and, via additional connection means 9, to the capillary 8. The arrangement was tested with an HPLC sample changer by the firm Wissenschaftliche Gerätebau, Dr. Knauer GmbH, Berlin. Further, the sample changer delivers an electric signal by which the data processing of the spectrometer

or other processes can be started. An automated analysis process is made possible by this arrangement as in the arrangement according to Fig. 1.

Fig. 4 shows an arrangement with a gas pressure pump 26. A higher pressure can be achieved in a simpler manner compared with a hose pump, as can a pulsation-free delivery of the liquid. The cylindrical low-pressure vessel 27 which is made of plastic is closed by a cover 28. The carrier liquid 30 can be introduced via the closable opening 29. Pressure gas is supplied to the vessel 27 via the pneumatic valve 31. In the simplest case, this is compressed air. The pressure monitoring is carried out via a manometer 32. Valve 33 is used for aeration. The carrier liquid 30 reaches the sample injection valve 18 via a 1/16" HPLC capillary of PEEK 34, wherein this manual valve can also be replaced in this case by an HPLC sample changer 23 with the installed valve 24 (Fig. 3). The liquid flows from the valve 18, 24 to the capillary 8 via connection means 9.

Testing was conducted using a simple commercial 2-liter or 4-liter liquid pressurized container of plastic as is used, for example, in ion chromatography for transporting washing liquids (DIONEX Corp., Sunnyvale, CA, USA, type 39163 or 39164, maximum pressure 10 psi (0.07 MPa)) and an own-construction gas pressure pump with a liquid reservoir of about 2.5 l and a maximum working pressure of 1 MPa, as is shown schematically in Fig. 4. A higher gas pressure is advantageous when low-pressure chromatography is to be operated by adding an analytic partition column between the valve 18, 24 and the capillary 8.

Fig. 5 is identical to the arrangement according to Fig. 2 with the exception of the utilized HPLC piston pump 35. The manual sample feed valve 18 can also be replaced by the HPLC sample changer 23 (Fig. 3) for purposes of automation. It is sensible to use an HPLC pump when the arrangement according to the invention is to be used simultaneously for an on-line HPLC partition in connection with element determination with greater detection.

Fig. 6 shows a section from Fig. 2, Fig. 4 or Fig. 5 in which an analytic partition or enrichment column 36 is added between the sample feed valve 18 and capillary 8, in addition. In this way, the manual valve 18 can likewise be replaced by the HPLC sample changer 23 (Fig. 3), so that automatic chromatography is possible.

Fig. 7 shows an embodiment form of the flame-heated tube 6. Due to the evaporation of aerosol droplets in the interior of the tube 6, the temperature decreases, particularly at higher sample flow rates, which has a disadvantageous effect on sensitivity and on the quantity of elements which can be determined by atomic absorption spectrometry. The temperature in the tube interior should be as high as possible for atomic absorption spectrometry measurements. The interior temperature of the tube can be appreciably increased when the tube has additional bore holes 38 oriented to the burner slit 3 apart from the inlet opening 7 and the openings at the two ends 37. A tested arrangement had six bore holes of 3 mm diameter each. Instead of bore holes, one (or more) slit(s) can also be provided for flame gases to enter. The flame gases serve not only to increase temperature, but additionally provide for a reducing atmosphere in the interior of the tube. The tube can also have additional bore holes 39 which are directed upward or openings of different shapes, e.g., slits, in order to achieve a partial transverse flow of flame gases through the tube. The tube can also be a quartz tube with two slits such as that used by Varian, Australia, for backing up flame gases. This quartz tube is provided with an additional lateral bore hole of 1.7 mm into which the capillary 8 projects.

The progress that can be achieved with an arrangement according to the invention is shown in two examples.

Using the example of cadmium determination with an arrangement corresponding to Fig. 2, Fig. 8 shows that an AAS signal which can be evaluated easily is obtained from small sample quantities, e.g., 2.5 μl, and with small concentrations at the same time. A cadmium concentration of 10 ng/ml was measured in each instance. A time axis in minutes is designated by 40. A sample volume of 2.5 μl results in signal 41, sample volumes of 10 μl, 50 μl and 200 μl result in signals 42, 43 and 44. The absolute sample quantity for generating the signal 41 is 25 pg.

Using the example of lead determination in water, Fig. 9 shows the gain in sensitivity compared with conventional flame AAS. A time axis in minutes is designated by 45. Signal 46 was obtained with an arrangement corresponding to Fig. 2, wherein the sample volume was 10 μl and lead concentration was 0.2 μg/ml. The signal for the measurement with conventional flame AAS of a sample with the

same lead concentration (0.2 μg/m) is designated by 47, wherein ten-times the sample volume (100μl) was used because of the large sample required by the conventional technique. The signal for a measurement with conventional flame AAS is designated by 48, wherein the sample volume was also 100 μl, but the lead concentration was increased ten-fold (2 μg/m). The signal 46 corresponds to an absolute lead quantity of 2 ng (10 μl sample volume with 0.2 μg/ml Pb). The signals 47 and 48 measured with conventional flame AAS correspond to an absolute lead quantity of 20 ng (100 μl sample volume with 0.2 μg/ml Pb) and 200 ng (100 μl sample volume with 2 μg/ml Pb).